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TITLE: Influences of ginsenosides Rb1 and Rg1 on reversible focal brain ischemia in rats
AUTHOR(S): Zhang, Ying-Ge; Liu, Tian-Pei
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AUTHOR(S): Chu, Guoxiang; Chen, Xiu
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Anti-lipid peroxidation and protection of ginsenosides against cerebral ischemia-reperfusion injuries in rats

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ABSTRACT The correlation between protective effect of ginsenosides $R_b + R_0$ and brain endogenously-derived prostacyclin synthesis, thromboxane A_2 formation and lipid peroxidation were estimated in rats. Ginsenosides $R_b + R_0$ 100 mg/kg iv 30 min before 4-vessel occlusion elevated 6-keto-$PGF_{1\alpha}$ level, declined thromboxane B_2 and brain edema formation, reduced the rise of lipid peroxides and suppressed the reduction in both creatine phosphokinase (CK) and superoxide dismutase (SOD) activities in brain tissue after 40-min ischemia followed by 1-h reperfusion. Furthermore, these improvements were partially abolished by pretreating with iv indomethacin. It is concluded that ginsenosides possess protective effect on cerebral ischemia-reperfusion injury of rats and ginsenosides $R_b + R_0$ are the active principles. The underlying mechanism of protection is ascribed partially or mainly to the facilitated synthesis and release of prostacyclin, reduced formation of thromboxane A_2 and inhibited generation of free radicals and subsequent lipid peroxidation.

KEY WORDS transient cerebral ischemia; reperfusion injury; prostaglandins X; thromboxane A_2; free radicals; lipid peroxides; brain edema; ginseng; saponins; indomethacin

Ginsenosides and their components $R_b + R_0$ have protective effects on myocardial ischemia and reperfusion injury both *in vivo* and *in vitro*[1,2] possibly via facilitating the synthesis and release of myocardial prostacyclin, inhibiting formation of thromboxane A_2 and suppressing free radical generation and subsequent lipid peroxidation[2,3]. Ginsenosides decreased brain lactate content during anoxia, lowered the vertebral artery resistance in dog and protected against scopolamine-induced amnesia in rats. These results indicate that ginsenosides may dilate cerebral arteries and maintain cerebral function and metabolism. Recently, we found that ginsenosides protected against acute cerebral ischemia and reperfusion injuries in rats[4] and manifestation of action agrees well with other investigations that *Panax notoginseng* protected against acute incomplete cerebral ischemia in rabbits[5]. However, the underlying mechanism of protection needs to be clarified. The aim of present investigation is to examine the possible beneficial action of ginsenosides $R_b + R_0$ on acute cerebral ischemia and reperfusion. In order to elucidate the mechanism of protection, correlation among the actions, prostacyclin synthesis and lipid peroxidation were also explored by means of interrupting the cyclooxygenase pathway of arachidonic acid metabolism with indomethacin.

MATERIALS

Ginsenosides components $R_b + R_0$ were extracted from the root of *Panax ginseng*

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CA Meyer. The *Panax* apogenoles of ginsenoside R_b and R_0 are panaxadiol and oleanolic acid, respectively. The purification of ginsenoside R_b from $R_b + R_0$ mixture is very difficult owing to their similar polarities. The proportion of R_b to R_0 is 24:1 and the physiological activity of R_0 is by far less potent than that of R_b. CK kits (Beckman), thiobarbituric acid (Fluka AG) and indomethacin (Sigma), pyrogallol (Zhun Yi Chemical Factory), ^{125}I-TXB_2 as well as ^{125}I-6-keto-PGF_{1a} RIA kits (Chinese Academy of Medical Sciences), RIA of TXB_2 and 6-keto-PGF_{1a} (KONTRON Gamma Counting System, Switzerland)

METHODS

Sprague-Dawley rats of both sexes weighing 233 ± SD 30 g were used. Acute cerebral ischemia was produced by ligating bilateral common carotid arteries (CCA) and electrocauterizing both vertebral arteries according to 4-vessel occlusion method[6,7]. An atraumatic arterial clasp was loosely placed around each CCA without interrupting carotid blood flow. A second incision was made at the nucha and both alar foramina of the first cervical vertebrae were exposed for electrocauterization of vertebral arteries. An electrocautery needle (0.5 mm od) was inserted through each alar foramen and both vertebral arteries were occluded by electrocauterization. After 10 min, both CCA were clasped. Perfusion following ischemia was re-established by removing carotid clasps.

35 rats were randomly allocated into 5 groups: A) sham-operation: B) reperfusion 1-h after 40-min ischemia: C) ginsenosides $R_b + R_0$ 100 mg/kg: D) indomethacin 5 mg/kg + ginsenosides $R_b + R_0$ 100 mg/kg: E) indomethacin 5 mg/kg. Ginsenosides and/or indomethacin was slowly iv 30 min prior to 4-vessel occlusion. Same volume of physiological saline was iv in group A.

Rats were killed by cervical dislocation at the end of the experiment. The pial vessels on brain surface were removed and the brain was stored at -20℃. About 100 mg forebrain of left hemisphere was excised and homogenized in ice-cold 20-fold phosphate buffered saline (pH 7.4). After 0.2 ml homogenate was taken for extraction and RIA of TXB_2 and 6-keto-PGF_{1a}, the remaining homogenate was centrifuged at 45000 × *g* for 30 min. The supernatant was used for assays of SOD[8], MDA[9] and CK[10]. Brain water content was estimated according to dry-wet weight method[11].

RESULTS

Ginsenosides $R_b + R_0$ on CK release The CK levels in brain at 1-h reperfusion following 40-min ischemia were higher in group C than that in group B (Tab 1). Group D markedly declined the CK activity in contrast with group C, but still significantly elevated as compared with group B. No significant discrepancy between group E and group B was seen.

Prostaglandins level As shown in Tab 1, 40-min ischemia followed by 1-h reperfusion elicited an increase in thromboxane metabolite TXB_2 as compared with control level while prostacyclin degraded metabolite 6-keto-PGF_{1a} rose, but not significantly higher than control values. In group C the 6-keto-PGF_{1a} increased while the TXB_2 level dropped dramatically when compared with group B. Ginsenosides administration ameliorated the ratio of 6-keto-PGF_{1a} and TXB_2 by more than 200% over control value (Tab 1). Indomethacin had a profound effect on the generation of arachidonate metabolites. As expected, the levels of both 6-keto-PGF_{1a} and TXB_2 were decreased by pretreatment with indomethacin in comparison with group C or group B.

Brain SOD activity and MDA content

Tab 1. Effects of ginsenosides on brain water content, brain creatine phosphokinase (CK) release, superoxide dismutase (SOD) activity, malondialdehyde (MDA) content, brain levels of 6-keto-$PGF_{1\alpha}$ and TXB_2 and the ratio of 6-keto-$PGF_{1\alpha}$/TXB_2 at 1-h reperfusion after 40-min ischemia in rats. $n=7$, except the number in parentheses, $\bar{x}\pm SD$. $^{**}P<0.05$, $^{***}P<0.01$ *vs* sham-operation; $^{\dagger}P>0.05$, $^{\dagger\dagger}P<0.05$, $^{\dagger\dagger\dagger}P<0.01$ *vs* reperfusion; $\ddagger P>0.05$, $\ddagger\ddagger P<0.05$, $\ddagger\ddagger\ddagger P<0.01$ *vs* ginsenosides; §§§$P<0.01$ *vs* indomethacin & ginsenosides.

Group	Water content (%)	CK (IU/g brain)	SOD (μg/g brain)	MDA (nmol/g brain)	6-keto-$PGF_{1\alpha}$ (ng/g wet wt)	TXB_2 (ng/g wet wt)	6-keto-$PGF_{1\alpha}$/TXB_2 Ratio
Sham-operation	77.2±0.6	549±62	44±4	212±26	32.2±6.0	9.9±2.5(6)	3.5±1.2(6)
Reperfusion	79.3±0.9***	404±57***	23±4***	315±12***	42.9±11.8(6)	13.5±1.7(6)**	2.8±0.7(5)
Ginsenosides (100 mg/kg)	77.9±0.7††	579±45†††	36±3†††	244±19†††	58.5±8.8(6)††	6.0±3.0†††	9.1±3.6(5)†††
Indomethacin + Ginsenosides (5 mg + 100 mg/kg)	78.5±0.6‡	501±22‡‡‡	28±3‡‡‡	283±21‡‡‡	37.5±8.6(4)‡‡	8.9±2.6†††	5.6±1.3(4)†††
Indomethacin (5 mg/kg)		432±26†	22±4†	319±31†	22.3±6.5†††	10.7±2.7‡‡	2.2±0.8§§§

During ischemia and reperfusion SOD activity decreased while MDA content increased (Tab 1). Ginsenosides pretreatment improved the reduction of SOD activity and suppressed the rising of MDA content in brain. Simultaneous administration with indomethacin and ginsenosides partially abolished the beneficial alterations of SOD activity and MDA content caused by either ginsenoside pretreatment. Indomethacin itself showed no pronounced effect with regard to SOD activity and MDA content.

Brain water content Similar to brain MDA content, the brain water content in the reperfused group was elevated in comparison with control group (Tab 1), while ginsenosides administration abbreviated sharp increase in brain water content. Indomethacin + ginsenosides administration 30 min prior to ischemia showed a tendency to blunt the decrease of brain water content by ginsenosides, but no significant difference between these 2 groups was found.

DISCUSSION

The present experiment demonstrated that ginsenosides R_b+R_0 exert protective effect on acute cerebral ischemia and reperfusion injury of rats and also indicated that R_b+R_0 components of ginsenosides are the active principles. Regarding substantially lower proportion and less potent biological activity of R_0 (oleanolic acid), it seems appropriate to accredit the protective effect to ginsenoside R_b. Furthermore, the data secured from this model favor the conclusion that the protective mechanism is prostacyclin-mediated anti-lipid peroxidation, as evidenced by the interference of protection with indomethacin on biochemical parameters. It has been well documented that the cyclooxygenase metabolites in the brain increase during reperfusion after an ischemic insult and these vasoactive products are postulated to play an important role in the pathogenesis of cerebral ischemia and reperfusion[12]. Prostacyclin, acting to prevent platelet aggregation and vasoconstriction, has been shown to be invariably effective in experimental ischemia models[13]. So it is rational to draw the conclusion from our results that ginsenosides protection is

closely correlated with its facilitation of PGI_2 synthesis and inhibition of TXA_2 formation.

PGI_2 was shown to inhibit generation of oxygen free radicals produced by FMLP (f-metleuphe)-activated polymorphonuclear-leukocytes (PMN) and human neutrophils[14] and this finding is reconciled to the recent report that PGI_2 protected ischemic reperfused myocardium in the dog by inhibition of neutrophil activation, and hence, suppressing free radical generation and anti-lipid peroxidation[15]. In the present experiment, ginsenosides significantly abbreviated the rising of lipid peroxides and improved the reduction of SOD activity, indicating that ginsenosides have beneficial effect on free radical reaction and lipid peroxidation. Since these actions can be partially abolished by cyclooxygenase inhibitor indomethacin, it points to that the anti-lipid peroxidation is PGI_2-related.

In addition, the increased endogenous brain leukotriene C_4 is considered to be the immediate stimulus to provoke ischemic cerebral edema and is associated more closely with brain edema formation than production of prostaglandins such as TXA_2[12]. In our experiment, considering the significant suppression of brain edema by ginsenosides, it is not unreasonable to speculate that either inhibition of lipoxygenase activity or counteraction of vasoactive lipoxygenase products may also contributes to the protection of ginsenosides against cerebral ischemia and reperfusion injury. In conclusion, our experiment demonstrate that ginsenosides $R_b + R_0$ can protect against experimental cerebral ischemia and reperfusion damage. The mechanism of action is mainly attributed to the facilitation of brain endogenous PGI_2 synthesis, reduction of TXA_2 formation and inhibition of free radical generation and subsequent lipid peroxidation. However, the protective mechanism has not been elucidated either exhaustively or conclusively. Whether there are other mechanisms involved in the protection await further investigations.

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人参皂甙抗脂质过氧化作用及对大鼠脑再灌注损伤的保护作用

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提要　用阻断 4 血管的方法造成大鼠急性脑缺血再灌注损伤。在缺血 40 min 后再灌 1 h 时人参皂甙 Rb+Ro 100 mg/kg iv 能显著保护脑组织 CK 及 SOD 活性，抑制脑水肿形成并减少 MDA 产生，而且能显著促进脑内 PGI_2 释放并抑制 TXA_2 合成。用吲哚美辛 5 mg/kg iv 能显著阻断 Rb+Ro 对脑缺血再灌注损伤的保护作用，这表明该保护作用是部分通过 PGI_2 中介的。

关键词　暂时性脑缺血；再灌注损伤；前列腺素X类；血栓素 A_2；游离基；过氧化脂质类；脑水肿；人参；皂甙；吲哚美辛

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维埃克斯的需氧代谢与混合功能氧化酶

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Aerobic metabolism of VX and mixed function oxidases

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ABSTRACT　In our preliminary study, it has been found that VX oxidase exists in the microsome fraction of rat liver and the catalytic reaction needs the participation of molecular oxygen and coenzyme I or II. In this paper, the data showed that deoxycholate inactivated both the mixed function oxidase and VX oxidase. The specific inhibitor proadifen of the mixed function oxidase also profoundly inhibited VX oxidase activity. The complex of VX and cytochrome P-450 exhibited typical difference spectrum of type I. Aniline competitively inhibited the inactivation of VX catalyzed by microsomes. These results indicate that VX is one of the substrates of mixed function oxidase. VX oxidase in the rat liver cells is exactly the mixed function oxidase.

KEY WORDS　mixed function oxidases; VX; aniline hydroxylase; spectrum analysis; proadifen; cholinesterase inhibitors; organophosphorus compounds

提要　脱氧胆酸钠可使混合功能氧化酶及 VX 氧化酶活性同时丧失，混合功能氧化酶的特异性抑制剂 proadifen 对 VX 氧化酶也有明显抑制作用，显示两种酶促反应的密切相关性。VX 与细胞色素 P-450 结合显示典型的 I 型差示光谱，苯胺可抑制微粒体对 VX 的酶促解毒，均表明 VX 是混合功能氧化酶的底物。大鼠肝脏细胞中的 VX 氧化酶就是混合功能氧化酶。

关键词　混合功能氧化酶类；维埃克斯；苯胺羟化酶；光谱分析；普罗地芬；胆碱脂酶抑制剂；有机磷化合物

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